

```
qiime demux emp-paired \  
  --m-barcodes-file AliyaMetaDataNew.tsv \  
  --m-barcodes-column BarcodeSequence \  
  --p-rev-comp-barcodes \  
  --p-rev-comp-mapping-barcodes \  
  --i-seqs emp-paired-end-sequences.qza \  
  --o-per-sample-sequences demux-full-new.qza \  
  --o-error-correction-details demux-details-new.qza
```

```
qiime demux summarize \  
  --i-data demux-full-new.qza \  
  --o-visualization demux-full-new.qzv
```

#####

```
qiime dada2 denoise-paired \  
  --i-demultiplexed-seqs demux-full-new.qza \  
  --p-trim-left-f 0 \  
  --p-trim-left-r 0 \  
  --p-trunc-len-f 195 \  
  --p-trunc-len-r 205 \  
  --o-table table.qza \  
  --o-representative-sequences rep-seqs.qza \  
  --o-denoising-stats denoising-stats.qza
```

```
qiime feature-table summarize \  
  --i-table table.qza \  
  --o-visualization table.qzv \  
  --m-sample-metadata-file AliyaMetaDataNew.tsv
```

```
qiime feature-table tabulate-seqs \  
  --i-data rep-seqs.qza \  
  --o-visualization rep-seqs.qzv
```

```
qiime metadata tabulate \  
  --m-input-file denoising-stats.qza \  
  --o-visualization denoising-stats.qzv
```

#####

```
qiime phylogeny align-to-tree-mafft-fasttree \  
  --i-sequences rep-seqs.qza \  
  --o-alignment aligned-rep-seqs.qza
```

```
--o-masked-alignment masked-aligned-rep-seqs.qza \  
--o-tree unrooted-tree.qza \  
--o-rooted-tree rooted-tree.qza
```

```
qiime diversity core-metrics-phylogenetic \  
--i-phylogeny rooted-tree.qza \  
--i-table table.qza \  
--p-sampling-depth 15500 \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--output-dir core-metrics-results
```

```
qiime diversity alpha-correlation \  
--i-alpha-diversity core-metrics-results/faith_pd_vector.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--o-visualization core-metrics-results/faith-pd-alpha-correlation.qzv
```

```
qiime diversity alpha-correlation \  
--i-alpha-diversity core-metrics-results/shannon_vector.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--o-visualization core-metrics-results/shannon-group-correlation.qzv
```

```
qiime diversity alpha-group-significance \  
--i-alpha-diversity core-metrics-results/faith_pd_vector.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--o-visualization core-metrics-results/faith-pd-group-significance.qzv
```

```
qiime diversity alpha-group-significance \  
--i-alpha-diversity core-metrics-results/evenness_vector.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--o-visualization core-metrics-results/evenness-group-significance.qzv
```

```
qiime diversity beta-group-significance \  
--i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--m-metadata-column Stagnation \  
--o-visualization core-metrics-results/unweighted-unifrac-stagnation-group-significance.qzv \  
--p-pairwise
```

```
qiime diversity beta-group-significance \  
--i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--m-metadata-column Material \  
--o-visualization core-metrics-results/unweighted-unifrac-material-group-significance.qzv \  
--p-pairwise
```

```
qiime diversity beta-group-significance \
  --i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file AliyaMetaDataNew.tsv \
  --m-metadata-column Stagnation \
  --p-method 'permdisp' \
  --o-visualization core-metrics-results/unweighted-unifrac-stagnation-permdisp.qzv \
  --p-pairwise
```

```
qiime diversity beta-group-significance \
  --i-distance-matrix core-metrics-results/unweighted_unifrac_distance_matrix.qza \
  --m-metadata-file AliyaMetaDataNew.tsv \
  --m-metadata-column Material \
  --p-method 'permdisp' \
  --o-visualization core-metrics-results/unweighted-unifrac-material-permdisp.qzv \
  --p-pairwise
```

```
qiime emperor plot \
  --i-pcoa core-metrics-results/unweighted_unifrac_pcoa_results.qza \
  --m-metadata-file AliyaMetaDataNew.tsv \
  --o-visualization core-metrics-results/unweighted-unifrac-emperor.qzv
```

```
qiime emperor plot \
  --i-pcoa core-metrics-results/bray_curtis_pcoa_results.qza \
  --m-metadata-file AliyaMetaDataNew.tsv \
  --o-visualization core-metrics-results/bray-curtis-emperor.qzv
```

#####

```
qiime feature-classifier classify-sklearn \
  --i-classifier gg-13-8-99-515-806-nb-classifier.qza \
  --i-reads rep-seqs.qza \
  --o-classification taxonomy.qza
```

```
qiime metadata tabulate \
  --m-input-file taxonomy.qza \
  --o-visualization taxonomy.qzv
```

```
qiime taxa barplot \
  --i-table table.qza \
  --i-taxonomy taxonomy.qza \
  --m-metadata-file AliyaMetaDataNew.tsv \
  --o-visualization taxa-bar-plots.qzv
```

```
qiime feature-table filter-samples \
```

```
--i-table table.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--p-where "[Wall]='W1'" \  
--o-filtered-table W1-table.qza
```

```
qiime composition add-pseudocount \  
--i-table W1-table.qza \  
--o-composition-table comp-W1-table.qza
```

```
qiime composition ancom \  
--i-table comp-W1-table.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--m-metadata-column Material \  
--o-visualization ancom-W1-Material.qzv
```

```
qiime taxa collapse \  
--i-table W1-table.qza \  
--i-taxonomy taxonomy.qza \  
--p-level 6 \  
--o-collapsed-table W1-table-l6.qza
```

```
qiime composition add-pseudocount \  
--i-table W1-table-l6.qza \  
--o-composition-table comp-W1-table-l6.qza
```

```
qiime composition ancom \  
--i-table comp-W1-table-l6.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--m-metadata-column Material \  
--o-visualization l6-ancom-W1-Material.qzv
```

```
#####
```

TESTING HERE AND BELOW

```
#####
```

```
qiime feature-table summarize \  
--i-table rarefied_table.qza \  
--o-visualization rarefied_table.qzv \  
--m-sample-metadata-file AliyaMetaDataNew.tsv
```

```
#####
```

THIS WORKS for filtering results

```
#####
```

```
qiime feature-table filter-samples \  
  --i-table table.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --p-where "[MonthlyFlush]='yes'" \  
  --o-filtered-table MonthFlush-filtered-table.qza
```

```
qiime feature-table summarize \  
  --i-table MonthFlush-filtered-table.qza \  
  --o-visualization MonthFlush-filtered-table.qzv \  
  --m-sample-metadata-file AliyaMetaDataNew.tsv
```

```
qiime feature-table filter-samples \  
  --i-table table.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --p-where "[Experiment]='Stagnation'" \  
  --o-filtered-table Stag-NoMonth0-filtered-table.qza
```

```
qiime feature-table summarize \  
  --i-table Stag-NoMonth0-filtered-table.qza \  
  --o-visualization Stag-NoMonth0-filtered-table.qzv \  
  --m-sample-metadata-file AliyaMetaDataNew.tsv
```

```
qiime feature-table filter-samples \  
  --i-table MonthFlush-filtered-table.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --p-where "[SampleType]='Sample'" \  
  --o-filtered-table MonthFlush-filtered-table-NoBlank.qza
```

```
qiime feature-table summarize \  
  --i-table MonthFlush-filtered-table-NoBlank.qza \  
  --o-visualization MonthFlush-filtered-table-NoBlank.qzv \  
  --m-sample-metadata-file AliyaMetaDataNew.tsv
```

```
qiime feature-table filter-samples \  
  --i-table Stag-NoMonth0-filtered-table.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --p-where "[SampleType]='Sample'" \  
  --o-filtered-table Stag-NoMonth0-filtered-table-NoBlank.qza
```

```
qiime feature-table summarize \  
  --i-table Stag-NoMonth0-filtered-table-NoBlank.qza \  
  --o-visualization Stag-NoMonth0-filtered-table-NoBlank.qzv \  
  --m-sample-metadata-file AliyaMetaDataNew.tsv
```

#####

```
qiime diversity core-metrics-phylogenetic \  
  --i-phylogeny rooted-tree.qza \  
  --i-table MonthFlush-filtered-table.qza \  
  --p-sampling-depth 20980 \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --output-dir core-metrics-results-MonthFlush
```

```
qiime diversity core-metrics-phylogenetic \  
  --i-phylogeny rooted-tree.qza \  
  --i-table Stag-NoMonth0-filtered-table-NoBlank.qza \  
  --p-sampling-depth 20980 \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --output-dir core-metrics-results-MonthFlush-NoMonth0
```

#####

#Random Forest

#####

```
qiime sample-classifier classify-samples \  
  --i-table MonthFlush-filtered-table.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --m-metadata-column StagType \  
  --p-optimize-feature-selection \  
  --p-parameter-tuning \  
  --p-estimator RandomForestClassifier \  
  --p-n-estimators 20 \  
  --p-test-size 0.2 \  
  --output-dir MonthFlush-classifier
```

```
qiime metadata tabulate \  
  --m-input-file MonthFlush-classifier/predictions.qza \  
  --o-visualization MonthFlush-classifier/predictions.qzv
```

```
qiime metadata tabulate \  
  --m-input-file MonthFlush-classifier/probabilities.qza \  
  --o-visualization MonthFlush-classifier/probabilities.qzv
```

```
qiime metadata tabulate \  
  --m-input-file MonthFlush-classifier/feature_importance.qza \  
  --o-visualization MonthFlush-classifier/feature_importance.qzv
```

```
qiime feature-table filter-features \  
  --i-table MonthFlush-filtered-table.qza \  
  --m-metadata-file MonthFlush-classifier/feature_importance.qza \
```

```
--o-filtered-table MonthFlush-classifier/important-feature-table.qza
```

```
qiime sample-classifier heatmap \  
  --i-table MonthFlush-filtered-table.qza \  
  --i-importance MonthFlush-classifier/feature_importance.qza \  
  --m-sample-metadata-file AliyaMetaDataNew.tsv \  
  --m-sample-metadata-column StagType \  
  --p-group-samples \  
  --p-feature-count 30 \  
  --o-filtered-table MonthFlush-classifier/important-feature-table-top-30.qza \  
  --o-heatmap MonthFlush-classifier/important-feature-heatmap.qzv
```

```
qiime sample-classifier heatmap \  
  --i-table MonthFlush-filtered-table.qza \  
  --i-importance MonthFlush-classifier/feature_importance.qza \  
  --m-sample-metadata-file AliyaMetaDataNew.tsv \  
  --m-sample-metadata-column StagType \  
  --m-feature-metadata-file taxonomy.qza \  
  --m-feature-metadata-column Taxon \  
  --p-group-samples \  
  --p-feature-count 30 \  
  --o-filtered-table MonthFlush-classifier/important-feature-table-taxonomy-top-30.qza \  
  --o-heatmap MonthFlush-classifier/important-feature-heatmap-taxonomy.qzv
```

```
#####  
#Stats for Monthly Flush  
#####
```

```
qiime diversity alpha-correlation \  
  --i-alpha-diversity core-metrics-results-MonthFlush/faith_pd_vector.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --o-visualization core-metrics-results-MonthFlush/faith-pd-alpha-correlation.qzv
```

```
qiime diversity alpha-correlation \  
  --i-alpha-diversity core-metrics-results-MonthFlush/shannon_vector.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --o-visualization core-metrics-results-MonthFlush/shannon-group-correlation.qzv
```

```
qiime diversity alpha-group-significance \  
  --i-alpha-diversity core-metrics-results-MonthFlush/observed_features_vector.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --o-visualization core-metrics-results-MonthFlush/observed_features-group-significance.qzv
```

```
qiime diversity alpha-group-significance \  
  --i-alpha-diversity core-metrics-results-MonthFlush/observed_features_vector.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --o-visualization core-metrics-results-MonthFlush/observed_features-group-significance.qzv
```

```
--i-alpha-diversity core-metrics-results-MonthFlush/evenness_vector.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--o-visualization core-metrics-results-MonthFlush/evenness-group-significance.qzv
```

```
qiime diversity beta-group-significance \  
  --i-distance-matrix core-metrics-results-MonthFlush/bray_curtis_distance_matrix.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --m-metadata-column StagType \  
  --o-visualization core-metrics-results-MonthFlush/bray_curtis-StagType-group-significance.qzv \  
 \  
  --p-pairwise
```

```
qiime diversity beta-group-significance \  
  --i-distance-matrix core-metrics-results-MonthFlush/jaccard_distance_matrix.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --m-metadata-column StagType \  
  --o-visualization core-metrics-results-MonthFlush/jaccard-StagType-group-significance.qzv \  
  --p-pairwise
```

```
qiime diversity beta-group-significance \  
  --i-distance-matrix core-metrics-results-MonthFlush/weighted_unifrac_distance_matrix.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --m-metadata-column StagType \  
  --o-visualization  
core-metrics-results-MonthFlush/weighted-unifrac-StagType-group-significance.qzv \  
  --p-pairwise
```

```
qiime diversity beta-group-significance \  
  --i-distance-matrix core-metrics-results-MonthFlush/unweighted_unifrac_distance_matrix.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --m-metadata-column StagType \  
  --o-visualization  
core-metrics-results-MonthFlush/unweighted-unifrac-StagType-group-significance.qzv \  
  --p-pairwise
```

ANCOM

```
qiime feature-table filter-samples \  
  --i-table MonthFlush-filtered-table.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --p-where "[Wall]='W1'" \  
  --o-filtered-table Month-Flush-W1-table.qza
```

```
qiime composition add-pseudocount \  
  --i-table Month-Flush-W1-table.qza \  
  --o-composition Month-Flush-W1-table.qza
```



```
--o-composition-table comp-Month-Flush-W1-table.qza
```

```
qiime composition ancom \  
  --i-table comp-Month-Flush-W1-table.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --m-metadata-column Material \  
  --o-visualization ancom-Month-Flush-W1-Material.qzv
```

```
qiime taxa collapse \  
  --i-table Month-Flush-W1-table.qza \  
  --i-taxonomy taxonomy.qza \  
  --p-level 6 \  
  --o-collapsed-table Month-Flush-W1-table-l6.qza
```

```
qiime composition add-pseudocount \  
  --i-table Month-Flush-W1-table-l6.qza \  
  --o-composition-table comp-Month-Flush-W1-table-l6.qza
```

```
qiime composition ancom \  
  --i-table comp-Month-Flush-W1-table-l6.qza \  
  --m-metadata-file AliyaMetaDataNew.tsv \  
  --m-metadata-column Material \  
  --o-visualization l6-ancom-Month-Flush-W1-Material.qzv
```

```
#####
```

```
#PieCrust2
```

```
#####
```

```
qiime picrust2 full-pipeline \  
  --i-table MonthFlush-filtered-table.qza \  
  --i-seq rep-seqs.qza \  
  --output-dir q2-picrust2_output \  
  --p-placement-tool sepp \  
  --p-threads 4 \  
  --p-hsp-method pic \  
  --p-max-nsti 2 \  
  --verbose
```

```
#####
```

```
# Trying to fix weird piecrust error
```

```
#####
```

```
qiime dada2 denoise-paired \  
  --i-demultiplexed-seqs demux-full-new.qza \  
  --p-trim-left-f 0 \  
  --p-trim-left-r 0
```

```
--p-trunc-len-f 195 \  
--p-trunc-len-r 205 \  
--o-table table-oldQiime.qza \  
--o-representative-sequences rep-seqs-oldQiime.qza \  
--o-denoising-stats denoising-stats-oldQiime.qza
```

```
qiime feature-table filter-samples \  
--i-table table-oldQiime.qza \  
--m-metadata-file AliyaMetaDataNew.tsv \  
--p-where "[MonthlyFlush]='yes'" \  
--o-filtered-table MonthFlush-filtered-table-oldQiime.qza
```